PATENT COOPERATION TREATY 09/403690

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
BN 25 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 98/02341	21/04/1998	22/04/1997
Applicant		
BAVARIAN NORDIC RESEARCH INSTITUTE A/S et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 7 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readble form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. -

☐ as suggested by the applicant.

☐ None of the figures.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 98/02341

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see FURTHER INFORMATION sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-10 (all partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 1 and Seq.ID 2 or using oligonucleotide primers with Seq.ID 1 and Seq.ID 2 in combination with the oligonucleotide with Seq. ID 18 wherein the oligonucleotide primers/probes are specific for a gene encoding heat labile toxin for the amplification of a DNA sequence characteristic for enterotoxigenic E.coli and Seq.ID 18 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

2. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 3 and Seq.ID 4 or using using oligonucleotide primers with Seq.ID 3 and Seq.ID 4 in combination with the oligonucleotide with Seq. ID 19 wherein the oligonucleotide primers/probes are specific for a gene encoding heat stabile toxin for the amplification of a DNA sequence characteristic for enterotoxigenic E.coli and Seq.ID 19 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

3. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 5 and Seq.ID 6 or using oligonucleotide primers with Seq.ID 5 and Seq.ID 6 in combination with the oligonucleotide with Seq. ID 20 wherein the oligonucleotide primers/probes are specific for a gene encoding heat stabile toxin for the amplification of a DNA sequence characteristic for enteroaggregative E.coli and Seq.ID 20 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

4. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 7 and Seq.ID 8 or using oligonucleotide primers with Seq.ID 7 and Seq.ID 8 in combination with the oligonucleotide with Seq. ID 21 wherein the oligonucleotide primers/probes are specific for the pCDV432 plasmid for the amplification of a DNA sequence characteristic for enteroaggregative E.coli and Seq.ID 21 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

5. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 9 and Seq.ID 10 or using oligonucleotide primers with Seq.ID 9 and Seq.ID 10 in combination with the oligonucleotide with Seq. ID 22 wherein the oligonucleotide primers/probes are specific for the inv-plasmid for the amplification of a DNA sequence characteristic for enteroinvasive E.coli and Seq.ID 22 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

6. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 11 and Seq.ID 12 or using oligonucleotide primers with Seq.ID 11 and Seq.ID 12 in combination with the oligonucleotide with Seq.ID 23 wherein the oligonucleotide primers/probes are specific for the EAF plasmid for the amplification of a DNA sequence characteristic for enteropathogenic E.coli and Seq.ID 23 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

7. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 13 and Seq.ID 14 or using oligonucleotide primers with Seq.ID 13 and Seq.ID 14 in combination with the oligonucleotide with

Seq. ID 24 wherein the oligonucleotide primers/probes are specific for the eae gene for the amplification of a DNA sequence characteristic for enteropathogenic E.coli and Seq.ID 24 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

8. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 15 and Seq.ID 16/17 or using oligonucleotide primers with Seq.ID 15 and Seq.ID 16/17 in combination with oligonucleotide with Seq. ID 25 wherein the oligonucleotide primers/probes are specific for the gene encoding shiga-like toxin sltI for the amplification of a DNA sequence characteristic for enterohemorrhagic E.coli and Seq.ID 25 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

9. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 27 and Seq.ID 28 or using oligonucleotide primers with Seq.ID 27 and Seq.ID 28 in combination with the oligonucleotide with Seq.ID 26 wherein the oligonucleotide primers/probes are specific for the gene encoding shiga-like toxin sltII for the amplification of a DNA sequence characteristic for enterohemorrhagic E.coli and Seq.ID 26 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/02341

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 08582 A (BERGERON MICHEL G ;OUELLETTE MARC (CA); ROY PAUL H (CA)) 21 March 1996 See pages 24-25 see the whole document ---	1-10
Y	EP 0 556 504 A (SHIMADZU CORP) 25 August 1993 see the whole document ---	1-10
Y	WO 96 12801 A (TEXAS A & M UNIVERSITY SYST ;UNIV TULANE (US); ARNTZEN CHARLES J () 2 May 1996 See oligonucleotides with Seq.ID 7 and 22. Seq.ID 7 shows 100% identity in 13 bp overlap with Seq.ID 2; Seq.ID 22 shows 100% identity in 15 bp overlap with Seq.ID 18 --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 February 1999	24. 05. 1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hagenmaier, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 669 399 A (SHIMADZU CORP) 30 August 1995 see the whole document	1-10
A	HEID C A ET AL: "REAL TIME QUANTITATIVE PCR" GENOME RESEARCH, vol. 6, no. 10, October 1996, pages 986-994, XP000642795 see the whole document	1-10
A	DATABASE MEDLINE JOURNAL OF DAIRY SCIENCE, January 1997 BATT: "MOLECULAR DIAGNOSTICS FOR DAIRY-BORNE PATHOGENS" XP002094257 see abstract	1-10
A	IBRAHIMI AND GENTZ: "A FUNCTIONAL INTERACTION BETWEEN THE SIGNAL PEPTIDE AND THE TRANSLATION APPARATUS IS DETECTED BY THE USE OF A SINGLE POINT MUTATION WHICH BLOCKS TRANSLOCATION ACROSS MAMMALIAN ENDOPLASMIC RETICULUM" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 262, no. 21, 1987, pages 10189-10194, XP002094256	1-10
A	& DATABASE EMBL AC: M17101, 1988 see abstract	1-10
P,Y	DATABASE EMBL AC: T97602; WO9737685, October 1997 * not prior art. XP002094258 Enterotoxigenic E.coli heat-labile (LT) PCR primer 12 has 100% identity to Seq.ID 2. see abstract	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 98/02341

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9608582 A	21-03-1996	AU 3468195 A	29-03-1996
		BR 9508918 A	21-10-1997
		CA 2199144 A	21-03-1996
		EP 0804616 A	05-11-1997
		JP 10504973 T	19-05-1998
		NO 971111 A	09-05-1997
		NZ 292494 A	25-03-1998
EP 0556504 A	25-08-1993	JP 2067556 C	10-07-1996
		JP 5227999 A	07-09-1993
		JP 7102158 B	08-11-1995
		US 5516898 A	14-05-1996
		US 5525718 A	11-06-1996
		US 5468852 A	21-11-1995
		JP 2067558 C	10-07-1996
		JP 5317098 A	03-12-1993
		JP 7102159 B	08-11-1995
WO 9612801 A	02-05-1996	AU 691707 B	21-05-1998
		AU 4194096 A	15-05-1996
		CA 2203679 A	02-05-1996
		EP 0793717 A	10-09-1997
		JP 10507916 T	04-08-1998
EP 0669399 A	30-08-1995	JP 7236500 A	12-09-1995
		JP 7284400 A	31-10-1995
		US 5795717 A	18-08-1998

09/403690

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* BN 25 PCT

Box No. I TITLE OF INVENTION

TaqMan-PCR for the detection of pathogenic E. coli strains

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

Bavarian Nordic Research Institute A/S
Naverland 2
DK-2600 Glostrup
Denmark

☐ This person is also inventor.

Telephone No. 00-45-4343-8444

Facsimile No. 00-45-4343-8999

Teleprinter No.

State *(i.e. country)* of nationality: DK

State *(i.e. country)* of residence: DK

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

Pfeffer, Klaus
Prinzregentenstr. 130
D-81677 München
Germany

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Dr. Petra Pielken
Bavarian Nordic Research Institute GmbH
Frankfurter Ring 193 a
D-80807 München

Telephone No. 00-49-(0)89-324752-19

Facsimile No. 00-49-(0)89-324752-44

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- ☒ **AP** **ARIPO Patent:** **GH** Ghana, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ☒ **AL** Albania .....
- ☒ **AM** Armenia .....
- ☒ **AT** Austria .....
- ☒ **AU** Australia .....
- ☒ **AZ** Azerbaijan
- ☒ **BA** Bosnia and Herzegovina .....
- ☒ **BB** Barbados
- ☒ **BG** Bulgaria .....
- ☒ **BR** Brazil .....
- ☒ **BY** Belarus
- ☒ **CA** Canada
- ☒ **CH and LI** Switzerland and Liechtenstein
- ☒ **CN** China .....
- ☒ **CU** Cuba .....
- ☒ **CZ** Czech Republic .....
- ☒ **DE** Germany .....
- ☒ **DK** Denmark .....
- ☒ **EE** Estonia .....
- ☒ **ES** Spain .....
- ☒ **FI** Finland .....
- ☒ **GB** United Kingdom
- ☒ **GE** Georgia .....
- ☒ **GH** Ghana .....
- ☒ **HU** Hungary .....
- ☒ **IL** Israel .....
- ☒ **IS** Iceland
- ☒ **JP** Japan .....
- ☒ **KE** Kenya .....
- ☒ **KG** Kyrgyzstan .....
- ☒ **KP** Democratic People's Republic of Korea .....
- ☒ **KR** Republic of Korea .....
- ☒ **KZ** Kazakstan .....
- ☒ **LC** Saint Lucia
- ☒ **LK** Sri Lanka
- ☒ **LR** Liberia
- ☒ **LS** Lesotho .....
- ☒ **LT** Lithuania
- ☒ **LU** Luxembourg .....
- ☒ **LV** Latvia
- ☒ **MD** Republic of Moldova .....
- ☒ **MG** Madagascar .....
- ☒ **MK** The former Yugoslav Republic of Macedonia .....
- ☒ **MN** Mongolia
- ☒ **MW** Malawi .....
- ☒ **MX** Mexico .....
- ☒ **NO** Norway
- ☒ **NZ** New Zealand .....
- ☒ **PL** Poland .....
- ☒ **PT** Portugal .....
- ☒ **RO** Romania
- ☒ **RU** Russian Federation .....
- ☒ **SD** Sudan
- ☒ **SE** Sweden
- ☒ **SG** Singapore
- ☒ **SI** Slovenia .....
- ☒ **SK** Slovakia .....
- ☒ **SL** Sierra Leone
- ☒ **TJ** Tajikistan .....
- ☒ **TM** Turkmenistan .....
- ☒ **TR** Turkey .....
- ☒ **TT** Trinidad and Tobago .....
- ☒ **UA** Ukraine .....
- ☒ **UG** Uganda .....
- ☒ **US** United States of America .....
- ☒ **UZ** Uzbekistan .....
- ☒ **VN** Viet Nam .....
- ☒ **YU** Yugoslavia .....
- ☒ **ZW** Zimbabwe

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☒ CY...Cyprus .....
- ☒ GM...Gambia .....
- ☒ GW...Guinea-Bissau .....
- ☒ ID...Indonesia .....
- ☐ .....

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____.

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country *(in which, or for which, the application was filed)*	Filing Date *(day/month/year)*	Application No.	Office of filing *(only for regional or international application)*
item (1) Denmark (DK)	22. April 1997 (22.04.1997)	0451/97	
item (2)			
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☐ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s) : _____

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA / _____

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:*

Country (or regional Office): Date *(day/month/year)*: Number:

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	3	sheets
2. description	48	sheets
3. claims	10	sheets
4. abstract	1	sheets
5. drawings		sheets
Total	62	sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☒ priority document(s) *identified in Box No. VI as item(s):*
5. ☒ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☒ nucleotide and/or amino acid sequence listing (diskette)
8. ☒ other *(specify)*: Remarks, 1 page

Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

München, den 20.04.1998

Petra Pielken

Dr. Petra Pielken

For receiving Office use only

1. Date of actual receipt of the purported international application:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority specified by the applicant: ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid

2. Drawings:
☐ received:
☐ not received:

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

Copy for the Elected Office (EO/US)

PCT/EP98/02341

PATENT COOPERATION TREATY

09/403690

50 50

PCT SP

From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

PIELKEN, Petra
Bavarian Nordic Research Institute GmbH
Fraunhoferstrasse 18B
D-82152 Martinsried
ALLEMAGNE

Date of mailing (day/month/year)
22 December 1999 (22.12.99)

Applicant's or agent's file reference
BN 25 PCT

IMPORTANT NOTIFICATION

International application No.
PCT/EP98/02341

International filing date (day/month/year)
21 April 1998 (21.04.98)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
BAVARIAN NORDIC RESEARCH INSTITUTE GMBH
Fraunhoferstrasse 18 b
D-82152 Martinsried
Germany

State of Nationality: DE
State of Residence: DE
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [X] the address [X] the nationality [X] the residence

Name and Address
BAVARIAN NORDIC RESEARCH INSTITUTE A/S
Naverland 2
DK-2600 Glostrup
Denmark

State of Nationality: DK
State of Residence: DK
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:
Form PCT/IB/306 issued on 09 October 1998 should be disregarded.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
Dominique DELMAS

Telephone No.: (41-22) 338.83.38

003027254

Form PCT/IB/306 (March 1994)

09/403690

PATENT COOPERATION TREATY

REC'D 30 JUL 1999
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
BN 25 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP98/02341	21/04/1998	22/04/1997
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant BAVARIAN NORDIC RESEARCH INSTITUTE A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 14 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03/11/1998	28.07.99
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. (+49-89) 2399-0 Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer Knudsen, H Telephone No. (+49-89) 2399

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP98/02341

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-48 as originally filed

Claims, No.:

1-17 as received on 14/07/1999 with letter of 12/07/1999

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-17 (partially).

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 1-17 (partially).

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-17
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-17 (NO)
Industrial applicability (IA)	Yes:	Claims	1-17
	No:	Claims	

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

ITEM I:

1. The sequence listings filed by the applicant were filed later than the application and therefore do not form part of the original application documents.

2. Amended claim 1 goes beyond the content of the original disclosure of the application. The original application does not disclose a method which uses a set of primer pairs capable of differentiating two groups of pathogenic E.coli, but a method, in which a primer pair, which hybridises to a specific gene characteristic for the E.coli group, is used. Moreover, the original application is directed to a method for detecting specific group-specific toxins (ie heat labile toxin etc.) of pathogenic E.coli, whereas present claim 1 is not limited to the detection of these specific toxins. Claim 1 therefore appears to contravene Art.34(2) PCT.

3. The same objection apply, mutatis mutandis, to claims 10, 13 and 16-17.

4. The original application does not appear to contemplate kits containing more than one primer pair (ie original claims 7-9 refer to **a** set of primers selected from the primer pairs mentioned in the claim). The sets which comprise two or more primer pairs or two or more labelled probes therefore do not appear to have a basis in the original application. Thus, claims 11-16 appear to contravene Article 34(2) PCT.

   The amendments introduced by the applicant are so fundamental that it would not be possible to examine the claims as if the amendments had not been made. Despite the above objections, the IPER is therefore drafted on the basis of the amended claims filed with letter of 12.07.1999.

ITEM III:

5. The present application has been searched and is examined, only insofar as the claims concern the detection of enterotoxigenic E.coli with primers having the sequences of SEQ. ID Nos. 1 & 2, optionally combined with the primer with SEQ ID NO.18.
   Consequently, only a partial examination of claims 1-17 have been carried out.

ITEM V:

NOVELTY:

6. EP-A-0 556 504 (D1) discloses oligonucleotides selectively hybridizable with specific genes of pathogenic microorganisms, eg the LT and the ST genes of toxigenic Escherichia coli. The oligonucleotides are used as primers for gene amplification by PCR and the result is determined by the length of the amplification products. It is suggested to use the method in the investigation of food poisoning.

   The subject-matter of claims 1-17 are distinguished therefrom in that at least two primer pairs are used. The subject-matter of these claims is therefore considered novel.

INVENTIVE STEP:

7. The applicant explains that the present claims are distinguished from D1 in that two pathogenic groups of enterobacteria are detected and that the primers must be selected to provide sufficient differentiation between the different groups and sufficient sensitivity against the sequence variants within each group.

   However, the IPEA is of the opinion that the use of another primer pair together with the primer pair, which consists of SEQ ID NOS.1 and 2, is obvious for the skilled person who would wish to detect all the well-known pathogenic groups. The combination of the detection of the gene encoding heat labile toxin with the detection of another target DNA in a sample can be carried out by routine methods. Moreover, the problems related to the identification of the primers and probes with the best combination of universality and specificity are well-known in the field and the selection of the primers on the basis of sequence data appear to be a matter of routine work for the skilled person.

   The detection of the heat labile toxin with a primer pair which consists of SEQ ID NOS. 1 & 2 is considered obvious for the following reasons:

The sequence of the heat labile toxin of enterotoxigenic E.coli is known in the art (cf D1 (p.11, l.21-22) and ref.84 cited in the present application). Nevertheless, though a primer overlapping with a part of the primer LT-2 of claim 2 is known from WO 96/12801 (D2) (cf Example 1 and SEQ ID NO.7), the prior art discloses neither the complete primer LT-2 nor a primer substantially overlapping with LT-1.

However, in the absence of any unexpected effects, the selection of a primer pair from within the known sequence encoding the heat labile toxin does not appear to involve an inventive step. The fact that the primers can be used for distinguishing the ETEC from other pathogenic E.coli does not appear to be surprising for the skilled person who knows that heat labile toxin is characteristic for ETEC. The applicant argues that the selection of primers involves an inventive step, because they must be capable of differentiating between the different groups of pathogenic E.coli and must bind universally to all strains of this group, but has not pointed to any special problems which have been overcome in the selection of the primers with SEQ ID NOS.1 and 2.

Claims 1-3, 10-12 and 17 therefore do not appear to be inventive.

8. WO 96/08582 (D3) discloses the detection of pathogens, eg E.coli, by a multiplex PCR method, eg the TaqMan method (cf p.25, l.10). The use of the TaqMan method in combination with primers specific for enterotoxigenic E.coli therefore does not involve an inventive step. Claims 4-6. 8 and 13-16 are therefore not considered inventive.

9. D2 discloses a primer used for producing a fragment which does not contain the bacteria signal sequence for the LT gene. The said primer shows an overlap with SEQ ID NO.18 (cf SEQ ID NO.22 and Example 21). For the skilled person who wishes to implement the TaqMan procedure in the detection of the heat labile protein from enterotoxigenic E.coli, it would appear to be obvious for the skilled person that the primer with SEQ ID NO.18 could be used as the fluorescently labelled probe. Claim 7 therefore does not appear to be inventive.

10. In the absence of an indication of a technical problem solved in an unobvious way by the reaction conditions mentioned in claim 9, the optimization of a PCR is considered an obvious task for the skilled person. Thus, claim 9 does not appear to involve an inventive step.

INDUSTRIAL APPLICABILITY:

11. All of the claims refer to chemical compounds or in-vitro assays and are therefore considered industrially applicable.

ITEM VI:

Certain published documents (Rule 70.10)

Document	*Publication date*
Database EMBL AC: T97602 identical to WO 97/37685	October 1997

12. The above document is published after the present application's priority date, but before its filing date. It is therefore relevant only for those parts of the present application, if any, which do not have a valid claim to priority.
Moreover, the above patent document may become relevant prior art in the Regional phase of the present application.

ITEM VIII:

13. The goal of the invention appears to be the provision of a method and primer pairs which specifically amplify sequences specific for each of the subgroups of pathogenic E.coli. However, this is not reflected in claim 1 which only refers to the use of a set of primer pairs allowing differentiation of at least two groups of pathogenic E.coli strains. If the present wording of claim 1 encompasses the situation, in which a primer pair is not capable of binding specifically to a single group of pathogenic E.coli in the presence of the other groups, then the present wording is inconsistent with the description.

14. The addition of the expression "using a set of oligonucleotide primer pairs allowing differentiation of at least two groups of pathogenic E.coli strains" to claim 1 introduces a lack of clarity. The use of a set of primer pairs implies that more than one primer pair is used, however, the differentiation of two pathogenic groups is an effect which could be expected from the use of a single primer pair. Claim 10 is considered to lack clarity for the same reasons.

15. Moreover, the examples in the present application shown in Tables 1-4 appear to indicate that the mere presence of the heat labile toxin is indicative of ETEC. It is therefore not completely clear whether the primers are specific for a specific heat labile toxin or whether the amplification of any heat labile toxin is characteristic for enterotoxigenic E.coli.

16. Contrary to the requirements of Rule 5(a)(ii) PCT, the closest prior art documents D1 and D3 are not identified in the description and the relevant background art disclosed therein is not briefly discussed.

International Patent Application: PCT/EP98/02341
Applicant: Bavarian Nordic Research Institute A/S
BN 25 PCT
July 12, 1999

New Claims

1. A method for detection of pathogenic enterobacteria in a sample comprising PCR amplification of DNA isolated from said sample using a set of oligonucleotide primer pairs allowing differentiation of at least two groups of pathogenic E.coli strains by amplification of a virulence factor/toxin gene characteristic for the respective group of the pathogenic E. coli strains.

2. The method according to claim 1 wherein the set of oligonucleotide primer pairs comprises two or more primer pairs selected from

- a primer pair that hybridises to a gene encoding heat labile toxin, or heat stabile toxin for amplification of a DNA sequence characteristic for enterotoxigenic E. coli;

- a primer pair that hybridises to a gene encoding heat stabile toxin for amplification of a DNA sequence characteristic for enteroaggregative E. coli;

- a primer pair that hybridises to the pCVD432 plasmid for amplification of a DNA sequence characteristic for enteroaggregative E.coli;

- a primer pair that hybridises to the inv-plasmid for amplification a DNA sequence contained in enteroinvasive E.coli;

- a primer pair that hybridises to the EAF plasmid, or the eae gene for amplification of a DNA sequence characteristic for enteropathogenic E.coli;

- a primer pair that hybridises to the genes encoding shiga-like toxin sltI or sltII for amplification of a DNA sequence characteristic for enterohemorrhagic E.coli.

3. The method according to claim 2 wherein

the primer pair that hybridises to the gene encoding heat labile toxin characteristic for enterotoxigenic E. coli is

LT-1: 5' GCG TTA CTA TCC TCT CTA TGT G 3' and
LT-2: 5' AGT TTT CCA TAC TGA TTG CCG C 3' ;

the primer pair that hybridises to the gene encoding heat stabile toxin characteristic for enterotoxigenic E. coli is

ST-1: 5′ TCC CTC AGG ATG CTA AAC CAG 3′ and
ST-2a: 5′ TCG ATT TAT TCA ACA AAG CAA C 3′ ;

the primer pair that hybridises for the gene encoding heat stabile toxin characteristic for enteroaggregative E. coli is

EASTI-1: 5′ AAC TGC TGG GTA TGT GGC TGG 3′ and
EASTI-2: 5′ TGC TGA CCT GCC TCT TCC ATG 3′ ;

the primer pair which hybridises to the pCVD432 plasmid is

EA-1: 5′ CTG GCG AAA GAC TGT ATC ATT G 3′ and
EA-2: 5′ TAA TGT ATA GAA ATC CGC TGT T 3′ ;

the primer pair which hybridises to the inv-plasmid is

EI-1: 5′ TTT CTG GAT GGT ATG GTG AGG 3′ and
EI-2: 5′ CTT GAA CAT AAG GAA ATA AAC 3′ ;

the primer pair which hybridises to the EAF plasmid is

EP-1: 5′ CAG GGT AAA AGA AAG ATG ATA AG 3′ and
EP-2: 5′ AAT ATG GGG ACC ATG TAT TAT C 3′ ;

the primer pair which hybridises to the eae gene is

EPeh-1: 5′ CCC GGA CCC GGC ACA AGC ATA AG 3′ and
EPeh-2: 5′ AGT CTC GCC AGT ATT CGC CAC C 3′ ;

the primer pair which hybridises to the gene encoding shiga-like toxin SltI is

SltI-1: 5′ ATG AAA AAA ACA TTA TTA ATA GC 3′ and
SltI-2: 5′ TCA CYG AGC TAT TCT GAG TCA AGC 3′;

the primer pair which hybridises to the gene encoding shiga-like toxin SltII is

SltII-1: 5′ ATG AAG AAG ATR WTT RTD GCR GYT TTA TTY G 3′ and
SltII-2: 5′ TCA GTC ATW ATT AAA CTK CAC YTS RGC AAA KCC 3′

wherein W is A/T, R is A/G, D is A/G/T, Y is C/T and K is G/T.

4. The method according to claims 1 to 3 wherein a polymerase having additional 5′-3′ exonuclease activity is used for the amplification of DNA, and an oligonucleotide probe labelled at the most 5′ base with a fluorescent dye and at the most 3′ base with a fluorescent quencher dye which hybridises within the target DNA is included in the amplification process; said labelled oligonucleotide probe being susceptible to 5′-3′ exonuclease degradation by said polymerase to produce fragments that can be detected by fluorogenic detection methods.

5. The method according to claim 4 wherein the labelled oligonucleotide probe is specific for the respective virulence factor/toxin gene to be detected.

6. The method according to claim 5 wherein

the labelled oligonucleotide probe is specific for the detection of heat labile toxin characteristic for enterotoxigenic E. coli;

the labelled oligonucleotide probe is specific for the detection of heat stabile toxin characteristic for enterotoxigenic E. coli;

the labelled oligonucleotide probe is specific for the detection of heat stabile toxin characteristic for enteroaggregative E. coli;

the labelled oligonucleotide probe is specific for the detection of pCVD432 plasmid;

the labelled oligonucleotide probe is specific for the detection of the inv-plasmid;

the labelled oligonucleotide probe is specific for the detection of the EAF-plasmid;

the labelled oligonucleotide probe is specific for the detection of the eae gene;

the labelled oligonucleotide probe is specific for the detection of shiga-like toxin SltI gene;

the labelled oligonucleotide probe is specific for the detection of shiga-like toxin SltII gene.

7. The method according to claim 6 wherein

the labelled oligonucleotide probe for the detection of heat labile toxin characteristic for enterotoxigenic E. coli is

5′ AGC TCC CCA GTC TAT TAC AGA ACT ATG 3′;

the labelled oligonucleotide probe for the detection of heat stabile toxin characteristic for enterotoxigenic E. coli is

5′ ACA TAC GTT ACA GAC ATA ATC AGA ATC AG 3′;

the labelled oligonucleotide probe for the detection of heat stabile toxin characteristic for enteroaggregative E. coli is

5′ ATG AAG GGG CGA AGT TCT GGC TCA ATG TGC 3′;

the labelled oligonucleotide probe for the detection of pCVD432 plasmid is

5′ CTC TTT TAA CTT ATG ATA TGT AAT GTC TGG 3′;

the labelled oligonucleotide probe for the detection of the inv-plasmid is

5′ CAA AAA CAG AAG AAC CTA TGT CTA CCT 3′

the labelled oligonucleotide probe for the detection of the EAF-plasmid is

5′ CTT GGA GTG ATC GAA CGG GAT CCA AAT 3′;

the labelled oligonucleotide probe for the detection of the eae gene is

5′ TAA ACG GGT ATT ATC AAC AGA AAA ATC C 3′;

the labelled oligonucleotide probe for the detection of shiga-like toxin SltI gene is

5′ TCG CTG AAT CCC CCT CCA TTA TGA CAG GCA 3′;

the labelled oligonucleotide probe for the detection of shiga-like toxin SltII gene is

5′ CAG GTA CTG GAT TTG ATT GTG ACA GTC ATT 3′.

8. The method according to claims 4 to 7 wherein the fluoroscent reporter dye is 6-carboxy-fluoroscein, tetrachloro-6-carboxy-fluoroscein, or hexachloro-6-carboxy-fluoroscein, and the fluorescent

quencher dye is 6-carboxytetramethyl-rhodamine.

9. The method according to claims 1 to 8 wherein the amplification process comprises 35 PCR cycles at a $MgCl_2$ concentration of 5.2 mmol, an annealing temperature of 55 °C and an extension temperature of 65 °C.

10. A set of primer pairs useful for PCR amplification of DNA of pathogenic enterobacteria allowing differentiation of at least two different groups of pathogenic E. coli strains by amplification of a virulence factor/toxin gene characteristic for the respective group of the pathogenic E.coli strains.

11. The set of primer pairs according to claim 10 comprising two or more primer pairs selected from

a primer pair that hybridises to a gene encoding heat labile toxin, or heat stabile toxin of enterotoxigenic E. coli;

a primer pair that hybridises to a gene encoding heat stabile toxin of enteroaggregative E. coli;

a primer pair that hybridises to the pCVD432 plasmid of enteroaggregative E. coli;

a primer pair that hybridises to the inv-plasmid of enteroinvasive E. coli;

a primer pair that hybridises to the EAF plasmid, or the eae gene of enteropathogenic E. coli;

a primer pair that hybridises to the gene encoding shiga-like toxin sltI or sltII of enterohemorrhagic E. coli.

12. The set of primer pairs according to claim 11 wherein

the primer pair which hybridises to the gene encoding heat labile toxin of enterotoxigenic E. coli is

LT-1: 5′ GCG TTA CTA TCC TCT CTA TGT G 3 and
LT-2: 5′ AGT TTT CCA TAC TGA TTG CCG C 3′ ;

the primer pair which hybridises to the gene encoding heat stabile toxin of enterotoxigenic E. coli is

ST-1: 5′ TCC CTC AGG ATG CTA AAC CAG 3′ and
ST-2a: 5′ TCG ATT TAT TCA ACA AAG CAA C 3′ ;

the primer pair which hybridises to the gene encoding heat stabile toxin of enteroaggregative E. coli is

EASTI-1: 5′ AAC TGC TGG GTA TGT GGC TGG 3′ and

EASTI-2: 5′ TGC TGA CCT GCC TCT TCC ATG 3′ ;

the primer pair which hybridises to the pCVD432 plasmid is

EA-1: 5′ CTG GCG AAA GAC TGT ATC ATT G 3′ and
EA-2: 5′ TAA TGT ATA GAA ATC CGC TGT T 3′ ;

the primer pair which hybridises to the inv-plasmid is

EI-1: 5′ TTT CTG GAT GGT ATG GTG AGG 3′ and
EI-2: 5′ CTT GAA CAT AAG GAA ATA AAC 3′ ;

the primer pair which hybridises to the EAF plasmid is

EP-1: 5′ CAG GGT AAA AGA AAG ATG ATA AG 3′ and
EP-2: 5′ AAT ATG GGG ACC ATG TAT TAT C 3′ ;

the primer pair which hybridises to the eae gene is

EPeh-1: 5′ CCC GGA CCC GGC ACA AGC ATA AG 3′ and
EPeh-2: 5′ AGT CTC GCC AGT ATT CGC CAC C 3′ ;

the primer pair which hybridises to the shiga-like toxin sltI gene is

SltI-1: 5′ ATG AAA AAA ACA TTA TTA ATA GC 3′ and
SltI-2: 5′ TCA CYG AGC TAT TCT GAG TCA AGC 3′ ;

the primer pair which hybridises to the shiga-like toxin sltII is

SltII-1: 5′ ATG AAG AAG ATR WTT RTD GCR GYT TTA TTY G 3′

and

SltII-2: 5′ TCA GTC ATW ATT AAA CTK CAC YTS RGC AAA KCC 3′

wherein W is A/T, R is A/G, D is A/G/T, Y is C/T and K is G/T.

13. A set of labelled oligonucleotide probes useful for detection of pathogenic enterobacteria by TaqMan™-PCR being specific for virulence factor/toxin genes of pathogenic E. coli strains.

14. The set of probes according to claim 13 comprising

a labelled oligonucleotide probe specific for the detection of heat labile toxin characteristic for enterotoxigenic E. coli;

a labelled oligonucleotide probe specific for the detection of heat stabile toxin characteristic for enterotoxigenic E. coli;

a labelled oligonucleotide probe specific for the detection of heat stabile toxin characteristic for enteroaggregative E. coli;

a labelled oligonucleotide probe specific for the detection of pCVD432 plasmid;

a labelled oligonucleotide probe specific for the detection of the inv-plasmid;

a labelled oligonucleotide probe specific for the detection of the EAF-plasmid;

a labelled oligonucleotide probe specific for the detection of the eae gene;

a labelled oligonucleotide probe specific for the detection of shiga-like toxin SltI gene;

a labelled oligonucleotide probe specific for the detection of shiga-like toxin SltII gene.

15. The set of probes according to claim 14 wherein

the labelled oligonucleotide probe for the detection of heat labile toxin characteristic for enterotoxigenic E. coli is

5' AGC TCC CCA GTC TAT TAC AGA ACT ATG 3';

the labelled oligonucleotide probe for the detection of heat stabile toxin characteristic for enterotoxigenic E. coli is

5' ACA TAC GTT ACA GAC ATA ATC AGA ATC AG 3';

the labelled oligonucleotide probe for the detection of heat stabile toxin characteristic for enteroaggregative E. coli is

5′ ATG AAG GGG CGA AGT TCT GGC TCA ATG TGC 3′;

the labelled oligonucleotide probe for the detection of pCVD432 plasmid is

5′ CTC TTT TAA CTT ATG ATA TGT AAT GTC TGG 3′;

the labelled oligonucleotide probe for the detection of the inv-plasmid is

5′ CAA AAA CAG AAG AAC CTA TGT CTA CCT 3′

the labelled oligonucleotide probe for the detection of the EAF-plasmid is

5′ CTT GGA GTG ATC GAA CGG GAT CCA AAT 3′;

the labelled oligonucleotide probe for the detection of the eae gene is

5′ TAA ACG GGT ATT ATC AAC AGA AAA ATC C 3′;

the labelled oligonucleotide probe for the detection of shiga-like toxin SltI gene is

5′ TCG CTG AAT CCC CCT CCA TTA TGA CAG GCA 3′;—

the labelled oligonucleotide probe for the detection of shiga-like toxin SltII gene is

5'CAG GTA CTG GAT TTG ATT GTG ACA GTC ATT 3'.

16. A kit useful for diagnosing an enterobacteria infection in samples derived from a living animal body, including a human, by TaqMan™-PCR method comprising a set of primer pairs according to claims 10 to 12 and a set of oligonucleotide probes according to claims 13 to 15.

17. Use of the method according to claims 1 to 9 for diagnosing an enterobacteria infection in a sample derived from a living animal body, including a human, or for the detection of an enterobacteria contamination of consumables, such as meat, milk and vegetables.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 10 December 1998 (10.12.98)	
International application No. PCT/EP98/02341	**Applicant's or agent's file reference** BN 25 PCT
International filing date (day/month/year) 21 April 1998 (21.04.98)	**Priority date** (day/month/year) 22 April 1997 (22.04.97)
Applicant PFEFFER, Klaus	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

03 November 1998 (03.11.98)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Dominique DELMAS
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Copy for the Elected Office (EO/US)

PCT/EP98/02341

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

PIELKEN, Petra
Bavarian Nordic Research Institute GmbH
Fraunhoferstrasse 18B
D-82152 Martinsried
ALLEMAGNE

Date of mailing (day/month/year)
10 December 1998 (10.12.98)

Applicant's or agent's file reference
BN 25 PCT

IMPORTANT NOTIFICATION

International application No.
PCT/EP98/02341

International filing date (day/month/year)
21 April 1998 (21.04.98)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address
PIELKEN, Petra
Bavarian Nordic Research Institute GmbH
Frankfurter Ring 193a
D-80807 München
Germany

State of Nationality:
State of Residence:
Telephone No.: +49-89-324-752-19
Facsimile No.: +49-89-324-752-44
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
PIELKEN, Petra
Bavarian Nordic Research Institute GmbH
Fraunhoferstrasse 18B
D-82152 Martinsried
Germany

State of Nationality:
State of Residence:
Telephone No.: +49-89-324-752-19
Facsimile No.: +49-89-324-752-44
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[X] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
Dominique DELMAS

Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

002386875

PATENT COOPERATION TREAT

09/403690

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
BN 25 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 98/02341	21/04/1998	22/04/1997
Applicant		
BAVARIAN NORDIC RESEARCH INSTITUTE A/S et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 7 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readble form.

   ☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. -

   ☐ as suggested by the applicant.

   ☐ because the applicant failed to suggest a figure.

   ☐ because this figure better characterizes the invention.

   ☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 98/ 02341

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see FURTHER INFORMATION sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-10 (all partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 1 and Seq.ID 2 or using oligonucleotide primers with Seq.ID 1 and Seq.ID 2 in combination with the oligonucleotide with Seq. ID 18 wherein the oligonucleotide primers/probes are specific for a gene encoding heat labile toxin for the amplification of a DNA sequence characteristic for enterotoxigenic E.coli and Seq.ID 18 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

2. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 3 and Seq.ID 4 or using using oligonucleotide primers with Seq.ID 3 and Seq.ID 4 in combination with the oligonucleotide with Seq. ID 19 wherein the oligonucleotide primers/probes are specific for a gene encoding heat stabile toxin for the amplification of a DNA sequence characteristic for enterotoxigenic E.coli and Seq.ID 19 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

3. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 5 and Seq.ID 6 or using oligonucleotide primers with Seq.ID 5 and Seq.ID 6 in combination with the oligonucleotide with Seq. ID 20 wherein the oligonucleotide primers/probes are specific for a gene encoding heat stabile toxin for the amplification of a DNA sequence characteristic for enteroaggregative E.coli and Seq.ID 20 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

4. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 7 and Seq.ID 8 or using oligonucleotide primers with Seq.ID 7 and Seq.ID 8 in combination with the oligonucleotide with Seq. ID 21 wherein the oligonucleotide primers/probes are specific for the pCDV432 plasmid for the amplification of a DNA sequence characteristic for enteroaggregative E.coli and Seq.ID 21 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

5. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 9 and Seq.ID 10 or using oligonucleotide primers with Seq.ID 9 and Seq.ID 10 in combination with the oligonucleotide with Seq. ID 22 wherein the oligonucleotide primers/probes are specific for the inv-plasmid for the amplification of a DNA sequence characteristic for enteroinvasive E.coli and Seq.ID 22 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

6. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 11 and Seq.ID 12 or using oligonucleotide primers with Seq.ID 11 and Seq.ID 12 in combination with the oligonucleotide with Seq.ID 23 wherein the oligonucleotide primers/probes are specific for the EAF plasmid for the amplification of a DNA sequence characteristic for enteropathogenic E.coli and Seq.ID 23 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

7. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 13 and Seq.ID 14 or using oligonucleotide primers with Seq.ID 13 and Seq.ID 14 in combination with the oligonucleotide with

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Seq. ID 24 wherein the oligonucleotide primers/probes are specific for the eae gene for the amplification of a DNA sequence characteristic for enteropathogenic E.coli and Seq.ID 24 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

8. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 15 and Seq.ID 16/17 or using oligonucleotide primers with Seq.ID 15 and Seq.ID 16/17 in combination with oligonucleotide with Seq. ID 25 wherein the oligonucleotide primers/probes are specific for the gene encoding shiga-like toxin sltI for the amplification of a DNA sequence characteristic for enterohemorrhagic E.coli and Seq.ID 25 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

9. Claims: 1-10 (all partially)

A method, set of primers/probes and the use of the method for the detection of pathogenic E.coli in a sample comprising PCR amplification of DNA isolated from said sample using oligonucleotide primers with Seq.ID 27 and Seq.ID 28 or using oligonucleotide primers with Seq.ID 27 and Seq.ID 28 in combination with the oligonucleotide with Seq.ID 26 wherein the oligonucleotide primers/probes are specific for the gene encoding shiga-like toxin sltII for the amplification of a DNA sequence characteristic for enterohemorrhagic E.coli and Seq.ID 26 comprises a labelled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by the polymerase used, to produce detectable fragments.

INTERNATIONAL SEARCH REPORT

rnational Application No
PCT/EP 98/02341

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 08582 A (BERGERON MICHEL G ;OUELLETTE MARC (CA); ROY PAUL H (CA)) 21 March 1996 See pages 24-25 see the whole document ---	1-10
Y	EP 0 556 504 A (SHIMADZU CORP) 25 August 1993 see the whole document ---	1-10
Y	WO 96 12801 A (TEXAS A & M UNIVERSITY SYST ;UNIV TULANE (US); ARNTZEN CHARLES J () 2 May 1996 See oligonucleotides with Seq.ID 7 and 22. Seq.ID 7 shows 100% identity in 13 bp overlap with Seq.ID 2; Seq.ID 22 shows 100% identity in 15 bp overlap with Seq.ID 18 --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 February 1999	24. 05. 1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hagenmaier, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 669 399 A (SHIMADZU CORP) 30 August 1995 see the whole document ---	1-10
A	HEID C A ET AL: "REAL TIME QUANTITATIVE PCR" GENOME RESEARCH, vol. 6, no. 10, October 1996, pages 986-994, XP000642795 see the whole document ---	1-10
A	DATABASE MEDLINE JOURNAL OF DAIRY SCIENCE, January 1997 BATT: "MOLECULAR DIAGNOSTICS FOR DAIRY-BORNE PATHOGENS" XP002094257 see abstract ---	1-10
A	IBRAHIMI AND GENTZ: "A FUNCTIONAL INTERACTION BETWEEN THE SIGNAL PEPTIDE AND THE TRANSLATION APPARATUS IS DETECTED BY THE USE OF A SINGLE POINT MUTATION WHICH BLOCKS TRANSLOCATION ACROSS MAMMALIAN ENDOPLASMIC RETICULUM" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 262, no. 21, 1987, pages 10189-10194, XP002094256	1-10
A	& DATABASE EMBL AC: M17101, 1988 see abstract ---	1-10
P,Y	DATABASE EMBL AC: T97602; WO9737685, October 1997 XP002094258 Enterotoxigenic E.coli heat-labile (LT) PCR primer 12 has 100% identity to Seq.ID 2. see abstract -----	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 98/02341

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9608582 A	21-03-1996	AU 3468195 A	29-03-1996
		BR 9508918 A	21-10-1997
		CA 2199144 A	21-03-1996
		EP 0804616 A	05-11-1997
		JP 10504973 T	19-05-1998
		NO 971111 A	09-05-1997
		NZ 292494 A	25-03-1998
EP 0556504 A	25-08-1993	JP 2067556 C	10-07-1996
		JP 5227999 A	07-09-1993
		JP 7102158 B	08-11-1995
		US 5516898 A	14-05-1996
		US 5525718 A	11-06-1996
		US 5468852 A	21-11-1995
		JP 2067558 C	10-07-1996
		JP 5317098 A	03-12-1993
		JP 7102159 B	08-11-1995
WO 9612801 A	02-05-1996	AU 691707 B	21-05-1998
		AU 4194096 A	15-05-1996
		CA 2203679 A	02-05-1996
		EP 0793717 A	10-09-1997
		JP 10507916 T	04-08-1998
EP 0669399 A	30-08-1995	JP 7236500 A	12-09-1995
		JP 7284400 A	31-10-1995
		US 5795717 A	18-08-1998